

WHAT IS CLAIMED IS:

1. A human MGDF polypeptide that
5 specifically promotes the growth and development of human megakaryocytes, substantially free from other human proteins.
2. A polypeptide according to Claim 1,
10 wherein said polypeptide comprises amino acids 22-172 of FIG. 11.
3. A polypeptide according to Claim 2,
15 wherein said polypeptide comprises amino acids 22-195 of FIG. 11.
4. A polypeptide according to Claim 3,
having the amino acid sequence of MGDF-2.
- 20 5. A polypeptide according to Claim 1, wherein said polypeptide comprises amino acids 22-353 of FIG. 11.
6. A polypeptide according to Claim 5,
25 having the amino acid sequence of MGDF-1.
7. A polypeptide according to Claim 2, which has an amino acid sequence of a member selected from the group consisting of MGDF-4, MGDF-5, MGDF-6, MGDF-7, and
30 MGDF-8.
8. A polypeptide according to Claim 1, wherein said polypeptide comprises amino acids 22-184 of
FIG. 11.

9. A polypeptide according to Claim 1, wherein said polypeptide further comprises the sequence Met-Lys at the N-terminus thereof.

5 10. A polypeptide according to Claim 1, wherein said polypeptide comprises amino acids 22-184 of FIG. 11 and further comprises the sequence Met-Lys at the N-terminus thereof.

10 11. A polypeptide according to Claim 1, wherein said polypeptide comprises amino acids 22-353 of FIG. 11 and further comprises the sequence Met-Lys at the N-terminus thereof.

15 12. A polypeptide according to Claim 1, further comprising amino acids 1-21 of FIG. 11.

20 13. An isolated polynucleotide encoding a human MGDF polypeptide.

14. An isolated polynucleotide according to Claim 13, which encodes a human MGDF polypeptide according to any of Claims 1-12.

25 15. An isolated polynucleotide according to Claim 14, which is a DNA sequence.

16. A DNA sequence according to Claim 14, which is a cDNA sequence.

30 17. A cDNA according to Claim 16, which has a sequence as shown in FIG 11 or 12.

35 18. A DNA vector comprising a DNA sequence according to Claim 14.

19. The vector of Claim 18 wherein said DNA sequence is operatively linked to an expression control DNA sequence.

5 20. A host cell stably transformed or transfected with a DNA sequence according to Claim 14.

21. A host cell according to Claim 20, which expresses said DNA sequence.

10 22. A method for producing a human MGDF polypeptide, said method comprising growing a host cell according to Claim 21 in a suitable nutrient medium and isolating said human MGDF polypeptide from said cell or
15 said nutrient medium.

23. A method for producing a human MGDF polypeptide according to Claim 22, wherein said host cell is *E. coli*.

20 24. A method for producing a human MGDF polypeptide according to Claim 22, wherein said host cell is *CHO*.

25 25. An antibody reactive with a human MGDF polypeptide.

26. A monoclonal antibody according to Claim 25.

30 27. A recombinant antibody according to Claim 25.

35 28. A pharmaceutical composition comprising a human MGDF polypeptide in association with a pharmaceutically acceptable carrier.

29. A pharmaceutical composition according to Claim 28 in aqueous solution.

5 30. A pharmaceutical composition according to Claim 28 in lyophilized form.

10 31. A method for treating a patient having a deficiency of a human MGDF polypeptide, which comprises administering an effective amount of a human MGDF polypeptide to said patient.

15 32. A method for treating a patient having thrombocytopenia, which comprises administering an effective amount of a human MGDF polypeptide to said patient.

20 33. A method according to Claim 32, wherein said condition is selected from the group consisting of aplastic anemia, idiopathic thrombocytopenia, and thrombocytopenia resulting from drug or radiation treatment.

25 34. A method for increasing the number of mature megakaryocytes in a patient in need thereof, which comprises administering to said patient an effective amount of a human MGDF polypeptide.

30 35. A method for increasing the number of platelets in a patient in need thereof, which comprises administering to said patient an effective amount of a human MGDF polypeptide.

35 36. An MGDF derivative comprising an MGDF protein connected to at least one water soluble polymer.

37. An MGDF derivative of Claim 36, wherein said MGDF protein is selected from the group consisting of MGDF-1, MGDF-2, MGDF-4, MGDF-11, MGDF-12, MGDF-13, MGDF-14, and MGDF-15.

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38. An MGDF derivative of Claim 36, wherein said MGDF protein is recombinantly produced in a bacterial cell.

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39. An MGDF derivative of Claim 36, wherein said water soluble polymer is pharmaceutically acceptable.

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40. An MGDF derivative of Claim 36, wherein said water soluble polymer is selected from the group consisting of dextran, poly(N-vinyl pyrrolidone), polyethylene glycols, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols, polyvinyl alcohols and mixtures thereof.

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41. An MGDF derivative of Claim 36, wherein said water soluble polymer is a polyethylene glycol.

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42. An MGDF derivative according to Claim 41, wherein said polyethylene glycol is a monomethoxy-polyethylene glycol.

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43. An MGDF derivative according to Claim 41, wherein said polyethylene glycol is attached to said MGDF protein by an acyl or an alkyl linkage.

44. Pegylated MGDF.

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45. A method for attaching a water soluble polymer to MGDF, wherein said water soluble polymer has a single reactive aldehyde group, said method comprising:

- 5 (a) reacting MGDF with a water soluble polymer under reductive alkylation conditions, at a pH sufficiently acidic to allow the α -amino group at the amino terminus of said MGDF to be reactive; and
- (b) isolating MGDF attached to at least
10 one water soluble polymer.

46. A method for attaching a water soluble polymer to MGDF according to Claim 45, which further comprises the step of (c) separating MGDF attached to at
15 least one water soluble polymer from unreacted molecules.

47. A method for attaching a water soluble polymer to MGDF, wherein said water soluble polymer has
20 a single reactive ester group, said method comprising:

(a) reacting MGDF with a water soluble polymer under conditions so that MGDF becomes attached to the water soluble polymer through an acyl linkage; and

25 (b) isolating MGDF attached to at least one water soluble polymer.

48. A method for attaching a water soluble polymer to MGDF according to Claim 47, which further
30 comprises the step of (c) separating MGDF attached to at least one water soluble polymer from unreacted molecules.

49. A method of Claim 45 or 47, wherein said
35 polymer is pharmaceutically acceptable.

50. A method of Claim 45 or 47, wherein said water soluble polymer is selected from the group consisting of dextran, poly(N-vinyl pyrrolidone), polyethylene glycols, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols and polyvinyl alcohols.

51. A method of Claim 45 or 47, wherein said water soluble polymer is polyethylene glycol.

52. A method of Claim 45 or 47 wherein said pH is between about 3 and about 9.

53. A method of Claim 45, wherein said reductive alkylation conditions involve the use of sodium cyanoborohydride as a reducing agent.

54. A method for attaching a polyethylene glycol molecule to MGDF, wherein said polyethylene glycol molecule has a single reactive aldehyde group, said method comprising:

(a) reacting said MGDF with said polyethylene glycol molecule under reductive alkylation conditions, at a pH sufficiently acidic to allow the α -amino group at the amino terminus of said MGDF to be reactive; and

(b) obtaining the pegylated MGDF.

55. A method for attaching a polyethylene glycol molecule to an MGDF molecule according to Claim 54, which further comprises the step of (c) separating pegylated MGDF from unreacted molecules.

56. A method of Claim 54, wherein said polyethylene glycol molecule has a molecular weight of about 2kDa to about 100kDa.

57. A pegylated MGDF product produced by the process of Claim 54.

5 58. A substantially homogeneous preparation of MGDF monopegylated at the α amino group at the N-terminus of said MGDF.

10 59. A preparation of Claim 58, wherein said MGDF is monopegylated with a polyethylene glycol having an average molecular weight of 5kDa to 50kDa.

15 60. A pharmaceutical composition comprising pegylated MGDF and a pharmaceutically acceptable diluent, adjuvant or carrier.

20 61. A pharmaceutical composition comprising:
(a) a substantially homogenous preparation of monopegylated MGDF, said monopegylated MGDF consisting of a polyethylene glycol having a molecular weight of 5kDa to 50kDa connected to an MGDF protein solely at the N-terminus thereof via an alkyl linkage; and (b) a pharmaceutically acceptable diluent, adjuvant or carrier.

25 62. A pegylated MGDF according to Claim 44, which has the amino acid sequence of amino acids 22-184 of FIG. 11.

30 63. A pegylated MGDF according to Claim 62, wherein the polyethylene glycol group is attached to the N-terminus thereof.

35 64. A pegylated MGDF according to Claim 63, wherein the polyethylene glycol group has an average molecular weight of 10 to 50 kilodaltons.

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65. A pegylated MGDF according to Claim 64,
which is produced in *E. coli*.

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66. Monopegylated human MGDF.

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